Data Evaluation Record on the Acute Toxicity of AE F084658 Technical (metabolite of Glufosinate Ammonium) to Algae, *Pseudokirchneriella subcapitata*

EPA MRID Number 48444812

Data Requirement:	EPA DP Barcode	345709
	EPA MRID	48444812
	EPA Guideline	850.5400

Test material: **AE F084658 Technical (metabolite of Glufosinate Ammonium)** **Purity:** 97.5% w/w
Common name
Chemical name: IUPAC disodium methylphosphinato-formate
CAS name
CAS No.
Synonyms

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental Inc. **Date:** 7/14/11

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 10/19/11

Primary Reviewer: Catherine Aubee **Signature:** C Aubee
Biologist, US EPA/OPP/EFED/ERBIV **Date:** 1 June 2012

EPA PC Code 128850

Date Evaluation Completed: 01-06-2012

CITATION: Sowig, P., and H. Gosch. 2000. Algal growth inhibition – *Pseudokirchneriella subcapitata* - AE F084658; substance, technical (Dinatrium salt of AE F130947; metabolite of Glufosinate-Ammonium AE F039866). Unpublished study performed and sponsored by Aventis CropScience GmbH, Frankfurt am Main, Germany. Study completed December 15, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to **AE F084658 Technical** at nominal concentrations of 0 (negative control), 9.8, 17.6, 31.2, 54.6, and 97.5 mg ai/L (adjusted for percent purity) under static conditions. Mean-measured concentrations were <LOQ (<2.21, control), 8.4, 18.1, 31.3, 55.7, and 94.8 mg ai/L.

The most sensitive endpoint could not be determined due to a lack of toxicity in this study, resulting in overall NOAEC and EC_{50} values of 94.8 and >94.8 mg ai/L, respectively.

The % growth inhibition of cell density in the treated algal culture as compared to the control ranged from -9 to 7%.

No phytotoxic effects were observed.

This toxicity study is classified as scientifically sound is classified as **acceptable**. It is consistent with the guideline requirement for a Tier II algal toxicity study using a glufosinate-ammonium transformation product (dinatrium salt of MPF).

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test procedure followed the guidelines of the Organization for Economic Cooperation and Development (OECD), Guideline No. 201: Alga, Growth Inhibition Test (1984); U.S. Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision J., Hazard Evaluation: Nontarget Plants §123-2: Growth and Reproduction of Aquatic Plants (Tier 2; EPA 540/9-82-020; 1982); and EU directive 92/69/EWG Annex Part C: Methods for the Determination of Ecotoxicity. C.3. Algal Growth Inhibition Test. The study methods and results were evaluated according to U.S. EPA OPPTS 850.5400: Algal Toxicity, Tiers I and II and OECD No. 201, and differences and/or similarities were described. One deficiency and deviations from OPPTS 850.5400 and OECD 201 were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

2. The physico-chemical properties of the test material were not reported; OECD guidelines suggest that this information be reported. OPPTS guidelines do not address this topic.

3. The pH of the control ranged from 8.0 to 10.3 and in the test solutions ranged from 7.8 to 10.2; OPPTS guidelines suggest a pH of 7.5 ± 0.2 for this algal species. Additionally, OECD guidelines suggest that the control pH not vary by more than 1.5 units.

The deficiency and deviations do not substantively impact the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice as adopted by the OECD Council on 26th November, 1997 [C(97)186/Final)] for implementation at the national level.

A. MATERIALS:

1. Test material	**AE F084658 Technical (metabolite of Glufosinate Ammonium)**
Description:	White powder
Lot No./Batch No. :	Not reported
Purity:	97.5% w/w
Stability of compound under test conditions:	Analytical verification yielded recoveries at time 0 ranging from 91 to 109%

of the nominal concentrations. At test termination (96 hours), recoveries ranged from 81 to 100% of nominal. The test material was stable under the test conditions.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of AE F084658 Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Green algae; *Pseudokirchneriella subcapitata* Korshikov

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 61.81
Source: In-house cultures originally obtained from the Collection of Algal Cultures, Institute of Plant Physiology, University of Gottingen, Gottingen, Germany
Age of inoculum: 4 days
Method of cultivation: Algae were cultivated in nutrient medium

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks ***Criteria***
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test (dilution water, temperature, agitation, photoperiod, and light intensity) Not reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	The test vessels were placed in a waterbath positioned on an electric shaker	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 300 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name		Control pH: 8.0-10.3

Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.9-8.1 9.3-10.3 Yes $NaHCO_3$ N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water 7.5 after aeration N/A Filtered by ultrafiltration, ion exchange, and a charcoal unit Not reported Not reported Not reported Not reported Not reported	The deionized water was used to create reagent grade water that was used to prepare the algal medium. *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test material (0.1 mg) was dissolved in nutrient medium to create a primary stock solution. The stock solution was shaken well and defined amounts were pipetted proportionally into the test flasks. Defined amounts of pre-culture were added into each of the corresponding graduated cylinders to obtain the final cell concentration and then filled to an exact volume with nutrient medium.	
Aeration or agitation	Agitation; 100 rpm	
Initial cells density	1 x 10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks ***Criteria***
<u>Number of replicates</u> Control: Solvent control: Treatments:	6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula</u> sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
<u>Test concentrations</u> Nominal (unadjusted for purity): Nominal (adjusted for purity): Measured:	0 (negative control), 10, 18, 32, 56, and 100 mg ai/L 0 (negative control), 9.8, 17.6, 31.2, 54.6, and 97.5 mg ai/L <LOQ (<2.21, control), 8.4, 18.1, 31.3, 55.7, and 94.8 mg ai/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples from all test levels and the control were analyzed via HPLC with UV detection (241 nm). Fortification samples were analyzed concurrently.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	24.5-25.6°C Continuous 62.44 ± 3.41 µE·m^{-2}·s^{-1} White spectrum fluorescent lamps of universal white-type	Dissolved oxygen: 8.2-9.6 mg/L Total hardness: 0.23 mmol/L Acid binding capacity: 0.22 mmol/L HCl/L Conductivity: 98 µS/cm *EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25ºC maintained at ± 2ºC and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- Cell density - Biomass - Growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell density and cell morphology were determined using counting chambers (Schreck, Hofheim, Germany) and a microscope (Zeiss, Oberkochen, Germany). The study author did not report how biomass and growth rate values were calculated.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None.	
Indicate whether there was an exponential growth in the control	Yes; cell density was 264 x 10^4 cells/mL at 96 hours.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At 96 hours, cell density in the negative control averaged 264 x 10^4 cells/mL, which yielded inhibitions of 7, -5, -7, -3, and -9% in the nominal 10, 18, 32, 56, and 100 mg ai/L test levels as compared to the control. An EC_{50} value was not calculated for this endpoint.

At 96 hours, biomass in the negative control averaged 7223 x 10^4 cells/mL*h, yielding inhibitions of -2, -3, -20, -4, and -14% as compared to the control. The 96-hour EC_{50} value was >100 mg ai/L.

At 96 hours, the growth rate in the negative control averaged 0.058 hours-1, yielding inhibitions of 1, -1, -1, -1, and -2%. The 96-hour EC_{50} value was >100 mg ai/L.

The overall NOAEC value, based on the level which had no significant effect on growth inhibition or cell morphology, was 100 mg ai/L.

No phytotoxic effects were observed.

Table 3: Effect of AE F084658 Technical on algal growth of *Pseudokirchneriella subcapitata*.

Treatment **Mean-measured (and nominal)** **mg ai/L**	**Initial cell Density (x 10^4 cells/mL)**	**Cell density (x 10^4 cells/mL) at**			
		24 hours	**48 hours**	**96 hours**	
				cell count	**% inhibition**
Negative control	1.0	5.4	43.0	264	N/A
8.4 (9.8)	1.0	5.5	21.7	244	7
18.1 (17.6)	1.0	5.7	22.8	278	-5
31.3 (31.2)	1.0	5.9	27.3	282	-7
55.7 (54.6)	1.0	5.7	20.9	273	-3
94.8 (97.5)	1.0	6.4	24.5	287	-9
Reference chemical (if used)	N/A				

Table 4: Effect of AE F084658 Technical on algal growth (*Pseudokirchneriella subcapitata*).

Treatment Mean-measured (and nominal) mg ai/L	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (hours^{-1})		Mean Biomass ($x\ 10^4$ cells/mL*h)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	1.0	0.0581	N/A	7223.2	N/A
8.4 (9.8)	1.0	0.0572	1	7374.4	-2
18.1 (17.6)	1.0	0.0586	-1	7466.4	-3
31.3 (31.2)	1.0	0.0588	-1	8686.4	-20
55.7 (54.6)	1.0	0.0584	-1	7483.2	-4
94.8 (97.5)	1.0	0.0590	-2	8257.6	-14

Table 5: Statistical endpoint values.*

Statistical Endpoint	Cell density	Growth rate	Biomass
NOAEC or EC_{05} (mg ai/L)	100	100	100
EC_{50} (mg ai/L)	ND	>100	>100
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A		

* Do not use this table, if the study was deemed unacceptable.

B. REPORTED STATISTICS:

The EC_{50} value for all endpoints could not be determined due to a lack of an inhibitory effect of ≥50%. The cell density data were not analyzed. The NOAEC was visually determined based on the concentration which had no significant effect on growth inhibition or growth rate or cell morphology. Initial nominal concentrations that were not adjusted for the percent purity of the test material were used for analysis.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Inhibitions for biomass and growth rate were <5%; therefore, the reviewer visually determined toxicity values for those endpoints. The reviewer tested cell density replicate data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Levene's test in Toxstat 3.5. The data met the assumptions of ANOVA, and were thus analyzed using the Bonferroni t-test and Williams' tests to determine the NOAEC. The ECx value could not be determined due to a lack of an inhibitory effect and a lack of statistically significant reductions relative to the control.

All toxicity values were determined using the 96-hour mean-measured concentrations. Cell density values were entered into Toxstat 3.5 as an abbreviated value, representing the value x 10^4.

Cell density
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement. However, the reviewer analyzed cell density and used mean-measured concentrations for analysis. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The experiment was initiated November 21, 1997, and was terminated November 25, 1997.

F. CONCLUSIONS:

This toxicity study is classified as scientifically sound is classified as **acceptable**. It is consistent with the guideline requirement for a Tier II algal toxicity study using a glufosinate-ammonium transformation product (dinatrium salt of MPF). The most sensitive endpoint could not be determined due to a lack of toxicity in this study, resulting in overall NOAEC and EC_{50} values of 94.8 and >94.8 mg ai/L, respectively.

Cell density
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Biomass
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Growth rate
EC_{05}: >94.8 mg ai/L 95% C.I.: N/A
EC_{50}: >94.8 mg ai/L 95% C.I.: N/A
NOAEC: 94.8 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

Organization for Economic Cooperation and Development, 1984. OECD Guidelines for Testing of Chemicals. Guideline No. 201: Alga, Growth Inhibition Test. 07 June 1984.

EU Directive 92/69/EWG Annex part C.3. Algae growth inhibition test; 29 Dec. 1992.

U.S. Environmental Protection Agency (EPA), 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants.

U.S. Environmental Protection Agency (EPA), 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792). Fed. Reg.,Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc. 1989. Release 6.08 TS 407. SAS Institute Inc., Cary, North Carolina 27511.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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Title:  AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File:             4812c             Transform:             NO TRANSFORMATION

                      Chi-Square Test for Normality
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                     Actual and Expected Frequencies

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             -----     -------------     -----------    -----------    -----
EXPECTED    1.4070        5.0820            8.0220         5.0820     1.4070
OBSERVED     1             5                 8              7          0

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     Chi-Square =   2.2500             (p-value = 0.6899)

     Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                           =  9.488  (alpha = 0.05 , df = 4)

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Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File:             4812c             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
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     D = 3420.9867
     W =   0.9066

     Critical W  = 0.8730 (alpha = 0.01 , N = 21)
              W  = 0.9080 (alpha = 0.05 , N = 21)

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Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File:             4812c             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
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   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5            280.8990          56.1798       0.4053

   Within (Error)     15           2079.3333         138.6222
--------------------------------------------------------------------------------
   Total              20           2360.2324
--------------------------------------------------------------------------------
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(p-value = 0.8377)

Critical F = 4.5556 (alpha = 0.01, df = 5,15)
= 2.9013 (alpha = 0.05, df = 5,15)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File: 4812c Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	3787.8857	757.5771	3.3217
Within (Error)	15	3420.9867	228.0658	
Total	20	7208.8724		

(p-value = 0.0323)

Critical F = 4.5556 (alpha = 0.01, df = 5,15)
= 2.9013 (alpha = 0.05, df = 5,15)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File: 4812c Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	Neg control	263.4667	263.4667		
2	8.4	243.9333	243.9333	1.8292	
3	18.1	277.4667	277.4667	-1.3110	
4	31.3	281.9333	281.9333	-1.7293	
5	55.7	272.4667	272.4667	-0.8428	
6	94.8	287.1333	287.1333	-2.2163	

Bonferroni t critical value = 2.6025 (1 Tailed, alpha = 0.05, df = 5,15)

Title: AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File: 4812c Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	8.4	3	27.7909	10.5	19.5333
3	18.1	3	27.7909	10.5	-14.0000

4	31.3	3	27.7909	10.5	-18.4667
5	55.7	3	27.7909	10.5	-9.0000
6	94.8	3	27.7909	10.5	-23.6667

Title: AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File: 4812c Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	263.4667	263.4667	269.9810
2	8.4	3	243.9333	243.9333	269.9810
3	18.1	3	277.4667	277.4667	269.9810
4	31.3	3	281.9333	281.9333	269.9810
5	55.7	3	272.4667	272.4667	269.9810
6	94.8	3	287.1333	287.1333	269.9810

Title: AE F084658 & P. subcapitata 96-hr cell density; mg ai/L
File: 4812c Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	263.4667				
8.4	269.9810	-0.6100		1.7500	k= 1, v=15
18.1	269.9810	-0.6100		1.8400	k= 2, v=15
31.3	269.9810	-0.6100		1.8700	k= 3, v=15
55.7	269.9810	-0.6100		1.8800	k= 4, v=15
94.8	269.9810	-0.6100		1.8900	k= 5, v=15

s = 15.1018

WARNING: Procedure has used isotonized means which differ from original (transformed) means.